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Medical Report

Type 2 Diabetes Mellitus (T2DM)

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The increasing incidence of obesity and Type 2 diabetes (T2D) confers significant burden on the US civilian and military healthcare system. It is hypothesized that the risk of future obesity and T2D can be mitigated by targeted interventions during early adult hood which are based on individual biological variability (e.g., personalized medicine using genetic biomarkers).

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INTRODUCTION

The prevalence of diabetes and obesity has increased nearly 50% over the past 20 years in the United States [1, 2]. Based on estimates from the Centers for Disease Control and Prevention (CDC), this trend will result in the prevalence of 1 in 3 Americans living with Type 2 diabetes (T2D) by the year 2050. Furthermore, the economic burden of T2D within the US is more than double when compared to those individuals with the absence of diabetes; a total of $174 billion in direct medical costs[1]. Nationally, the average age of onset of T2D decreased from 52 years in 1988 to 46 years in 2000 [3]. The Military Healthcare System (MHS) is not immune from these national trends. Every year, the Air Force Medical Service provides medical treatment for 47,000 patients diagnosed with T2D and diabetics and 100,000 who are considered to have "prediabetes"[4]. Among MHS beneficiaries ages 40 – 49, the prevalence of obesity (e.g., body mass index > 30kg/m^3) has been recently reported to be 20% for active duty/guard and over 40% for retirees [2]. Furthermore, in 2011 it was reported that 13% of the Air Force active-duty population were obese; a significant risk factor for onset of T2D [5].

The contribution of heredity to the risk of obesity and T2D has been well-characterized in the scientific literature. Genome-Wide Association Studies (GWAS) have made significant progress in identifying genetic markers (e.g., single nucleotide polymorphisms or SNPs) that confer the risk of obesity and onset of T2D. The risk conferred by an individual's genetic "background" may explain why the active duty population continues to have weight-management challenges and increasing numbers of active-duty service members progressing on to develop T2D in spite of high fitness standards. Integrating the presence of an individual's genetic risk for T2D and obesity has the potential to translate into an evidence-based personalized medical approach through early detection, intervention, lifestyle modifications, and disease prevention/management programs.

The objective of this study was to evaluate the prevalence of genetic risk factors within the MHS beneficiary population (Air Force active duty, retirees, and military dependents). The specific aims were as follows:

Specific aim 1: Compare the prevalence of pre-identified SNPs among Tricare beneficiaries being treated for T2D and non-diseased "controls";

Specific aim 2: Identify genetic disparities in risk in subgroups stratified by age, gender and race;

Specific aim 3: Assess the prevalence of these risk-conferring genotypes among the "seemingly healthy" military student population.

METHODS

Single Nucleotide Polymorphisms (SNPs) selected for Study

GWAS have identified several SNPs that confer risk for T2D [6]. For this study, we selected 17 SNPs that are shown in Table 1.

Table 1: SNPs selected for study

SNP	Associated Gene	Chromosome
rs10923931	NOTCH2	1
rs7578597	THADA	2
rs864745	JAZF1	7
rs7961581	TSPAN8	12
rs7903146	TCF7L2	10
rs13266634	SLC30A8	8
rs4402960	IGFBP2	3
rs1801282	PPARG	3
rs1111875	HHEX	10
rs5219	KCNJ11	11
rs7754840	CDKAL1	6
rs10811661	CDKN2A/2B	9
rs8050136	FTO	16
rs9939609	FTO2	16
rs10010131	WFS1	4
rs646776	CELSR2	1
rs11591147	PCSK9	1

Although the exact cellular mechanistic pathways remain to be elucidated, the SNPs selected for this study influence β-cell function, insulin secretion, and lipid levels (Table 2).

Table 2: Mechanism of associated gene

Gene	Risk Allele	Mechanism
NOTCH2 (Neurogenic locus notch homolog protein 2 gene)	T	β-cell dysfunction.[9] Critical role in fetal pancreatic development.[19]
THADA (Thyroid adenoma associated)	T	Impairment of pancreatic β-cell function [9]
JAZF1 (JAZF zinc finger 1 gene)	A	β-cell dysfunction[9] Encodes a nuclear function as a transcriptional repressor. [17]
TSPAN8 (Tetraspanin-8, transmembrane 4 superfamily)	C	Impairment of pancreatic β-cell function [9]
TCF7L2 (Transcription factor 7-like 2 gene)	T	Involved in the growth, differentiation, proliferation, and insulin secretion of pancreatic β-cells.[13]

SLC30A8 (Solute carrier family 30 (zinc transporter), member 8 gene)	C	Associated with impaired β-cell functions.[9] Encodes a zinc transporter expressed in the secretory vesicles of beta-cells in the pancreas. [21]
IGF2BP2 (Insulin-like growth factor 2 mRNA binding protein 2)	T	Impairment of pancreatic β-cell function [8,9]
PPARG **(**Peroxisome proliferator-activated receptor gamma)	C	Insulin sensitivity [8, 9]
HHEX (Hematopoietically expressed homeobox)	G	Impairment of pancreatic β-cell function [8]
KCNJ11 (Potassium inwardly-rectifying channel, subfamily J, member 11 gene)	T	β-cell dysfunction / deficiency/defect in insulin secretion.[9] Potassium channel that is part of the sulfonylurea receptor complex is associated with regulation of insulin secretion.[18]
CDKAL1 (CDK5 regulatory subunit-associated protein 1-like 1)	C	Impairment of pancreatic β-cell function [8, 9]
CDKN2A/2B (Cyclin-dependent kinase inhibitor 2A)	T	Impairment of pancreatic β-cell function [8]
FTO (Fat mass and obesity associated gene)	A	Predisposition to diabetes through an effect on BMI. Common variation was reproducibly associated with BMI and obesity[12]
FTO2 (Fat mass and obesity associated gene)	A	Predisposition to diabetes through an effect on BMI. Common variation was reproducibly associated with BMI and obesity[12]
WSF1 (Wolfram syndrome 1 gene)	G	Critical for survival and function of insulin-producing pancreatic β-cells[22]
CELSR2 (Cadherin EGF LAG seven-pass G-type receptor 2 gene)	C	Associated with low-density lipoprotein cholesterol.[16]
PCSK9 (proprorein convertase)	T	Regulatory role in cholesterol homeostasis [11]

Study Populations

Three study groups (Military Trainees, T2D patients, and non-diabetics) were assessed for the prevalence of the SNPs listed in tables 1 and 2. Blood samples and relevant medical and

demographic information were collected from each subject under an active Wilford Hall Ambulatory Surgical Center (WHASC) IRB protocol. The sites of study recruitment were the WHASC Diabetes Center of Excellence (DCoE), the Lackland Air Force Base Reid Clinic, and the Kelly Clinic. Any DCoE patient who as 18 years or older and had been diagnosed with T2D by a health care provider was considered for enrollment into the study. Since one of our study aims sought to assess the age-specific trends of risk conferred by genetic background, we also sought enrollees from the retired military community. Our study's "control" population was also recruited from WHASC and was selected from Air Force active-duty, Air Force retired, and military dependents who had not been diagnosed with diabetes and were otherwise healthy. The Military Trainees for our third specific aim were recruited from the LAFB Trainee Health Clinic and were either generally healthy active but seeking care for follow-up or injury appointments.

Data Collected

Patient information included age, gender, race/ethnicity, smoking history, familial disease history, T2D complications, age of diagnosis, and relevant medications. Clinical information included blood pressure, weight, height, hemoglobin A1C, and fasting glucose.

Laboratory Genotyping

DNA was extracted from whole blood using the Maxwell 16 and subsequently analyzed by polymerase chain reaction (PCR) using Applied Biosystems TaqMan Universal PCR Master Mix and SNP Genotyping Assay Mix. Molecular assays were performed on Applied Biosystems 7500 Real-time PCR Systems.

Whole blood from the patient's sample was pipetted from the vacutainer tube into a purification vial from the MagNA Pure Compact Nucleic Acid Isolation Kit, and the vial was placed into the MagNA Pure Compact robotic instrument (Roche, Applied Science). Once loaded onto the instrument, the cells are disrupted and cellular proteins are digested by the addition of Lysis Buffer and Proteinase K. The released DNA was bound to the surface of magnetic glass particles. The cellular debris was removed by extensive washing and the purified DNA released from the magnetic beads by elution at higher temperatures.

The Taq polymerase used for this analysis was isolated from the temperature-resistant bacteria *Thermophilus aquaticus* and included in the TaqMan Universal PCR Master Mix (Applied Biosystems). The reaction mixture included 2X TaqMan Universal PCR Master Mix, 20X SNP Genotyping Assay Mix (Table 3), and 1-20ng of purified genomic DNA. Negative controls composed of 2X TaqMan Universal PCR Master Mix, 20X SNP Genotyping Assay Mix, and distilled water were run on each 96-well reaction plate.

The presence of a known T2D-associated genetic marker was identified by PCR on extracted, purified genomic DNA. Allelic Discrimination assays were performed, classifying unknown samples as homozygotes (samples having only allele 1 or allele 2) or heterozygotes (samples having both allele 1 and allele 2). In SNP genotyping, a single mismatch between probe and target sequences needs to be discriminated during PCR. Therefore, competing probes were designed to support specific and strong oligonucleotide binding. Each probe is labeled with a fluorescent dye so that the probes provided fluorescent signals at different wavelengths. The

fluorophore fluoresced at the appropriate wavelength and was read on the thermocycler. For the purposes of this study, the AB 7500 system was used for Real-Time PCR allelic discrimination assays. The amplification run was programmed to hold at 95^{o}C for 10 minutes followed by 40 cycles between 92^{o}C for 15 seconds and 600^{o}C for 1 minute.

Table 3. The 20X SNP genotyping assay mix used for this study

SNP	Associated Gene	ABI Assay ID number
rs7754840	CDKAL1	C_29246232_10
rs10811661	CDKN2A/2B	C_31288917_10
rs8050136	FTO	C_2031259_10
rs9939609	FTO 2	C_30090620_10
rs1111875	HHEX	C_11214581_10
rs4402960	IGFBP2	C_2165199_10
rs864745	JAZF1	C_7601116_20
rs5219	KCNJ11	C_11654065_10
rs10923931	NOTCH2	C_1188816_10
rs11591147	PCSK9	C_2018188_10
rs1801282	PPARG	C_1129864_10
rs646776	CELSR2	C_3160062_10
rs13266634	SLC30A8	C_357888_10
rs7903146	TCF7L2	C_29347861_10
rs7578597	THADA	C_32653841_10
rs7961581	TSPAN8	C_121473_10
rs10010131	WFS1	C_30473796_10

Statistical Analyses

Demographic and clinical characteristics as well as active-duty status and SNP prevalence were reported as descriptive statistics. To determine the risk conferred by SNPs towards T2D, we used logistic regression with the results expressed as the odds ratio. An odds ratio > 1 is interpreted as "increased risk" of diabetes diagnosis; an odds ratio < 1 is interpreted as an "inverse risk" of diabetes diagnosis. At the time of this study, the Principal Investigator elected to report significant odds ratios at both the 95% and 90% confidence level. The latter was to demonstrate "trending towards significance" of specific SNPs that have been shown to be significant in studies with a larger enrollment population.

RESULTS

The demographic characteristics and active duty status of our study's enrollees are provided in Table 4. Males in the T2D appeared to be more likely to enroll in this study whereas the inverse was observed among the non-diseased controls (CT). The race distribution between the T2D and CT was comparable among various categories. Finally, the difference in mean age between the T2D group and CT group was significant ($p = 0.01$).
When examining the clinical characteristics of the T2D and CT groups, we found body mass index (kg/m^3) and self-reported rates of smoking to be relatively comparable. Furthermore, blood pressure and resting heart rates were within clinical acceptable ranges in both the T2D and CT groups.

Table 4. Demographic characteristics and active-duty status among study enrollees

	Study Groups*		
Variable	CT	DM	MS
Gender, n (%)			
Female	251 (51.12)	366 (43.73)	68 (25.56)
Male	240 (48.88)	471 (56.27)	198 (74.44)
Total	491	837	266
AD Status, n (%)			
AD	218 (45.23)	44 (5.26)	0 (0)
Dependent	150 (31.12)	351 (41.94)	0 (0)
Military Trainee	0 (0)	0 (0)	264 (100)
Retired	99 (20.54)	428 (51.14)	0 (0)
N/A	15 (3.11)	14 (1.67)	0 (0)
Total	482	837	264
Race, n (%)			
American Indian	2 (0.42)	8 (0.97)	1 (0.38)
Asian	17 (3.56)	18 (2.19)	2 (0.76)
Black	81 (16.95)	185 (22.48)	39 (14.77)
Pacific Islander	2 (0.42)	1 (0.12)	1 (0.38)
White	360 (75.31)	597 (72.54)	221 (83.71)
Other	16 (3.35)	14 (1.7)	0 (0)
Total	478	823	264
Age			
N	467	823	264
Mean (SD)	46.57 (11.31)	59.09 (11.45)	21.32 (2.93)

* Study Groups: CT=Control, DM=Diabetic, MS=Military Student

We further sought to examine the frequency distribution of the selected 17 SNPs among the CT. T2D, and the Military Student population. Using conventional genotyping nomenclature, we categorized the SNP frequencies as follows: no SNP = wild type (WT), one SNP = heterozygous (WT/Mu) and two SNPs = homozygous (Mu). The distribution frequencies are listed in Table 6 on next page. We found that among Military students, the proportion of individuals possessing one or 2 copies of SNPs that confer risk of T2D diagnosis (*CDKL1, CELST2/ PSRC1, IGFBP2, JAZF1, and PPAR-γ*) were comparable to our study group who had been diagnosed with T2D.

Table 5. Distribution frequencies and proportions

Variable	Control			Type 2 diabetes patients			Military student		
	Wt	Wt/Mu	Mu	Wt	Wt/Mu	Mu	Wt	Wt/Mu	Mu
CDKAL1	210 (43.21)	237 (48.77)	39 (8.02)	352 (41.71)	389 (46.09)	103 (12.2)	101 (38.26)	131 (49.62)	32 (12.12)
CDKN2A_s_CDKN2B	354 (72.84)	120 (24.69)	12 (2.47)	646 (76.54)	189 (22.39)	9 (1.07)	194 (73.48)	66 (25)	4 (1.52)
CELSR2_s_PSRC1	293 (60.29)	172 (35.39)	21 (4.32)	505 (59.83)	292 (34.6)	47 (5.57)	150 (56.82)	103 (39.02)	11 (4.17)
FTO_1	200 (41.15)	218 (44.86)	68 (13.99)	328 (38.86)	400 (47.39)	116 (13.74)	105 (39.77)	127 (48.11)	32 (12.12)
FTO_2	200 (41.15)	218 (44.86)	68 (13.99)	328 (38.86)	400 (47.39)	116 (13.74)	105 (39.77)	127 (48.11)	32 (12.12)
HHEX	195 (40.12)	211 (43.42)	80 (16.46)	381 (45.14)	351 (41.59)	112 (13.27)	106 (40.15)	115 (43.56)	43 (16.29)
IGFBP2	234 (48.15)	188 (38.68)	64 (13.17)	400 (47.39)	345 (40.88)	99 (11.73)	129 (48.86)	106 (40.15)	29 (10.98)
JAZF1	163 (33.54)	227 (46.71)	96 (19.75)	256 (30.33)	398 (47.16)	190 (22.51)	77 (29.17)	125 (47.35)	62 (23.48)
KCNJ11	247 (50.82)	182 (37.45)	57 (11.73)	407 (48.22)	324 (38.39)	113 (13.39)	148 (56.06)	95 (35.98)	21 (7.95)
NOTCH2	373 (76.75)	109 (22.43)	4 (0.82)	608 (72.04)	222 (26.3)	14 (1.66)	196 (74.24)	64 (24.24)	4 (1.52)
PCSK9	473 (97.33)	13 (2.67)		808 (95.73)	35 (4.15)	1 (0.12)	256 (96.97)	8 (3.03)	
PPARG	401 (82.51)	78 (16.05)	7 (1.44)	709 (84)	84 (9.95)	51 (6.04)	206 (78.03)	34 (12.88)	24 (9.09)
SLC30A8	270 (55.56)	179 (36.83)	37 (7.61)	466 (55.21)	300 (35.55)	78 (9.24)	151 (57.2)	87 (32.95)	26 (9.85)
TCF7L2	249 (51.23)	195 (40.12)	42 (8.64)	394 (46.68)	383 (45.38)	67 (7.94)	127 (48.11)	116 (43.94)	21 (7.95)
THADA	332 (68.31)	147 (30.25)	7 (1.44)	629 (74.53)	209 (24.76)	6 (0.71)	194 (73.48)	68 (25.76)	2 (0.76)
TSPAN8_s_LGR5	283 (58.23)	182 (37.45)	21 (4.32)	502 (59.48)	319 (37.8)	23 (2.73)	166 (62.88)	87 (32.95)	11 (4.17)
WFS1	205 (42.18)	213 (43.83)	68 (13.99)	359 (42.54)	387 (45.85)	98 (11.61)	107 (40.53)	121 (45.83)	36 (13.64)

We next sought to assess the relationship between T2D diagnosis and the presence of any of the 17 selected SNPs (e.g., either one or two SNPs). We found that three SNPs demonstrated a relationship with T2D diagnosis irrespective of gender, or race (Figure 1). A SNP in *NOTCH2* (a gene associated with β-cell dysfunction) conferred an increased risk of T2D diagnosis (odds ratio/OR =1.3). However, a SNP in *HHEX* (a gene that is associated with insulin sensitivity) and *THADA* (a gene shown to affect the 2-hour insulin level during an oral glucose tolerance test) each conferred an inverse risk of T2D diagnosis (OR = 0.81, OR = 0.74, respectively). When we re-examined this risk within the population 30 – 55 years of age, we found that the inverse risk conferred by a SNP in either *HHEX* (OR = 0.68) or in *THADA* (OR = 0.68) was sustained while the increased risk conferred from a SNP in NOTCH2 was no longer statistically significant.

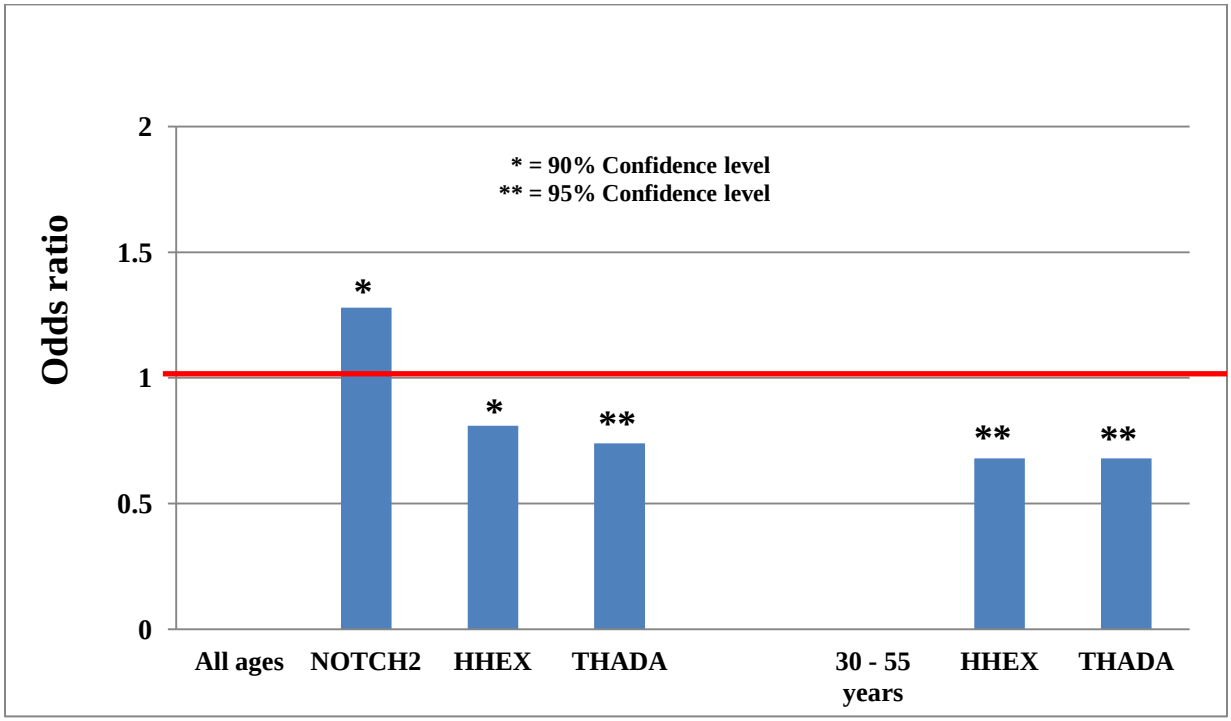

Figure 1. Genetic risk of T2D

We also explored whether there were gender-specific trends in genetic risk towards T2D diagnosis. This is based on published studies that report more females are more likely to be diagnosed with T2D than males. However, to date a biologically-plausible explanation for such is lacking. In our study we found that gender-specific risks are conferred by four of the SNPs listed in Table 1. We found that a SNP in *PCSK9* (a gene that plays a critical role in low-density lipoprotein clearance) conferred a 2-fold risk of T2D diagnosis among males of all ages (OR = 2.3) and over a 3-fold risk among males 30 – 55 years of age (OR = 3.32). The aforementioned inverse risk conferred by a SNP in *HHEX* was sustained among males 30-55 years of age (OR = 0.56) but not among females. Additionally, we found that a SNP in the *THADA* gene conferred an inverse risk towards T2D diagnosis among all females (OR = 0.72) as well as among females 30 – 55 years of age (OR = 0.58).

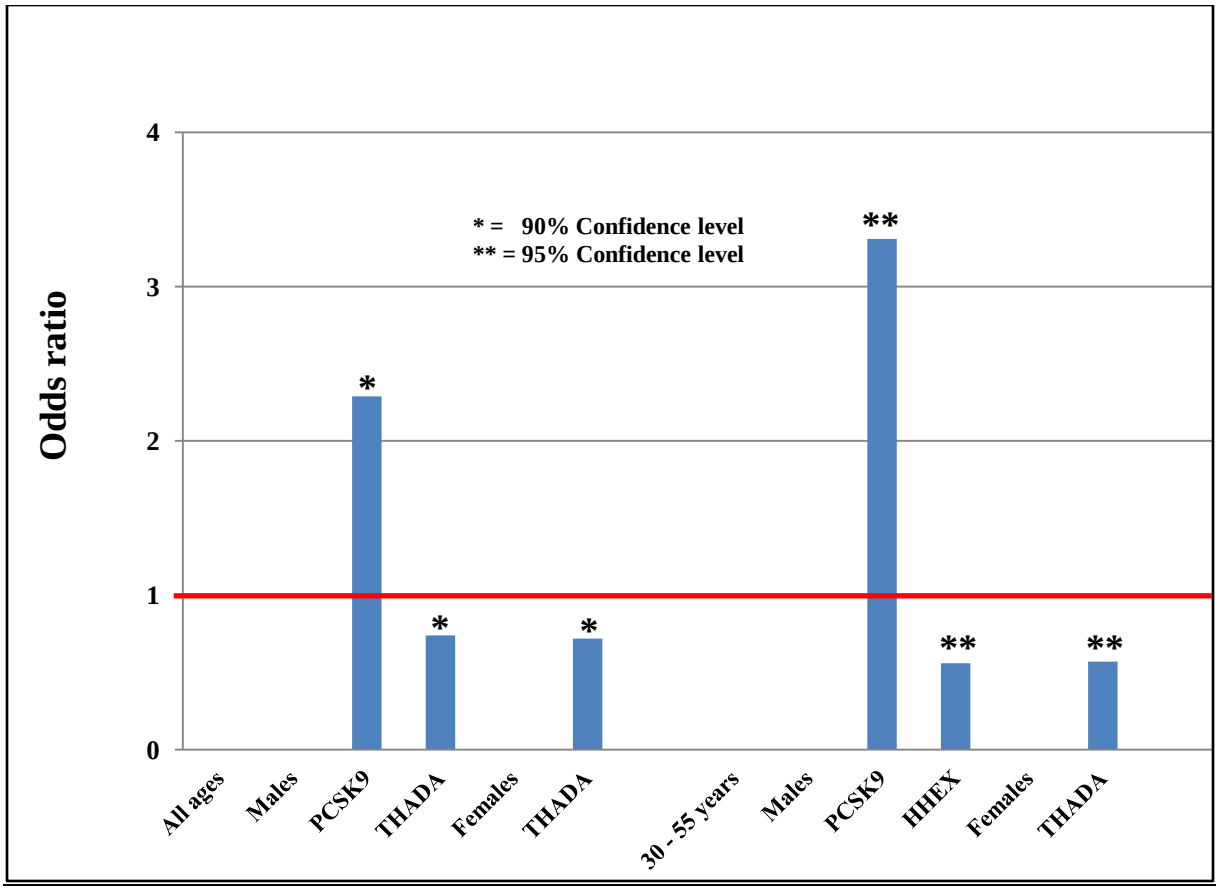

Figure 2. Gender-specific trends in genetic risk of T2D

Race and ethnic disparities regarding risk of T2D and associated complications have been well-studied. We found an association between six SNPs and T2D diagnosis when we stratified our population of all ages into the two largest race categories; White and Black. In Table 3a, we show that among Whites, a SNP in *HHEX*, *PPAR-g*, or *THADA* demonstrated an inverse risk of T2D diagnosis (OR=0.74, OR= 0.75 and OR = 0.58, respectively). *PPAR-g* is a SNP of interest to diabetes research since it influences the patient's response to a class of anti-diabetes drugs known as glitazones. The inverse risk conferred by a SNP in *THADA* continued to be observed among Blacks of all ages (OR = 0.58). When examining increased risk among Blacks, a 2-fold risk of T2D diagnosis was conferred by a SNP in either *SLC30A8* (OR = 2.05) or *JAZF1* (OR = 2.2). *SLC30A8* and *JAZF1* are each hypothesized to play critical roles in insulin secretion from the B-cells in the pancreas to the bloodstream, production of proteins that are important in the glucose metabolic pathway, and possibly β-cell dysfunction.

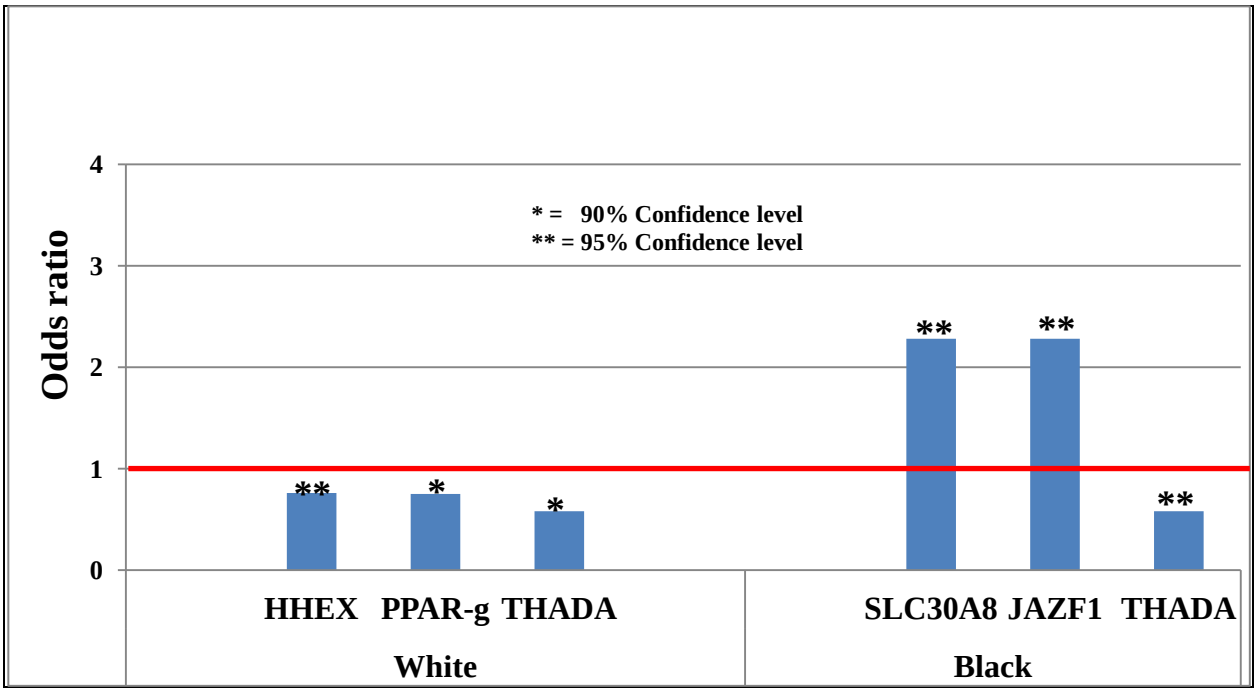

Figure 3a. Race-specific trends in genetic risk of T2D (all ages)

In Table 3b, we further report that among Blacks 30 – 55 years of age, the risk of diabetes diagnosis conferred by a SNP in *SLC30A8* increased substantially (OR = 4.31) while the risk conferred by *JAZF1*was comparable (OR = 2.08). Among Whites 30 – 55 years of age, the inverse risk conferred by a SNP in either the *HHEX (OR = 0.60) or THADA* (OR = 0.67) was sustained.

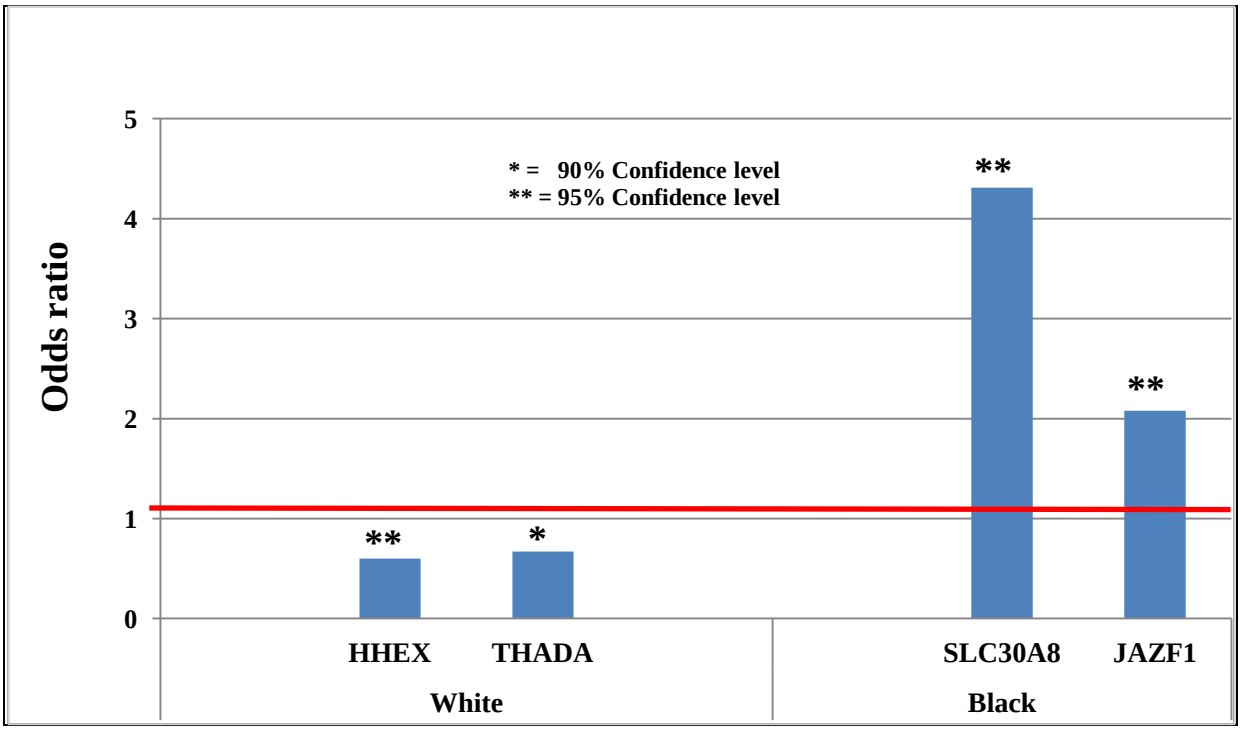

Figure 3b. Race-specific trends in genetic risk of T2D (30-55 years of age)

Our last set of analysis sought to assess the additive effect of obesity on the relationship between T2D diagnosis and our SNPs of interest. Reflecting guidelines from the Centers for Disease Control and Prevention, we defined obese as a body mass index (BMI) > = 30kg/m^3. The inverse risk associated with a SNP in *THADA* continued to be observed irrespective of age but only among obese individuals (OR = 0.61). The inverse risk associated with a SNP in *HHEX* was maintained significance (OR = 0.53) irrespective of age but only among the group that was not obese (BMI < 30 kg/m^3). An additional SNP in the gene known as *CELSR2/PSRC1* was found to confer an increased risk of T2D diagnosis (OR = 1.63). However, this finding was restricted to the group that was not obese and was within the 30 – 55 year age group. *CELSR2/PSRC1* has become increasingly important in research regarding diabetes management since it is associated with increasing levels of low-density lipoproteins in the serum; a powerful risk factor for coronary artery disease and a clinical indicator of a common co-morbidity among patients being treated for T2D.

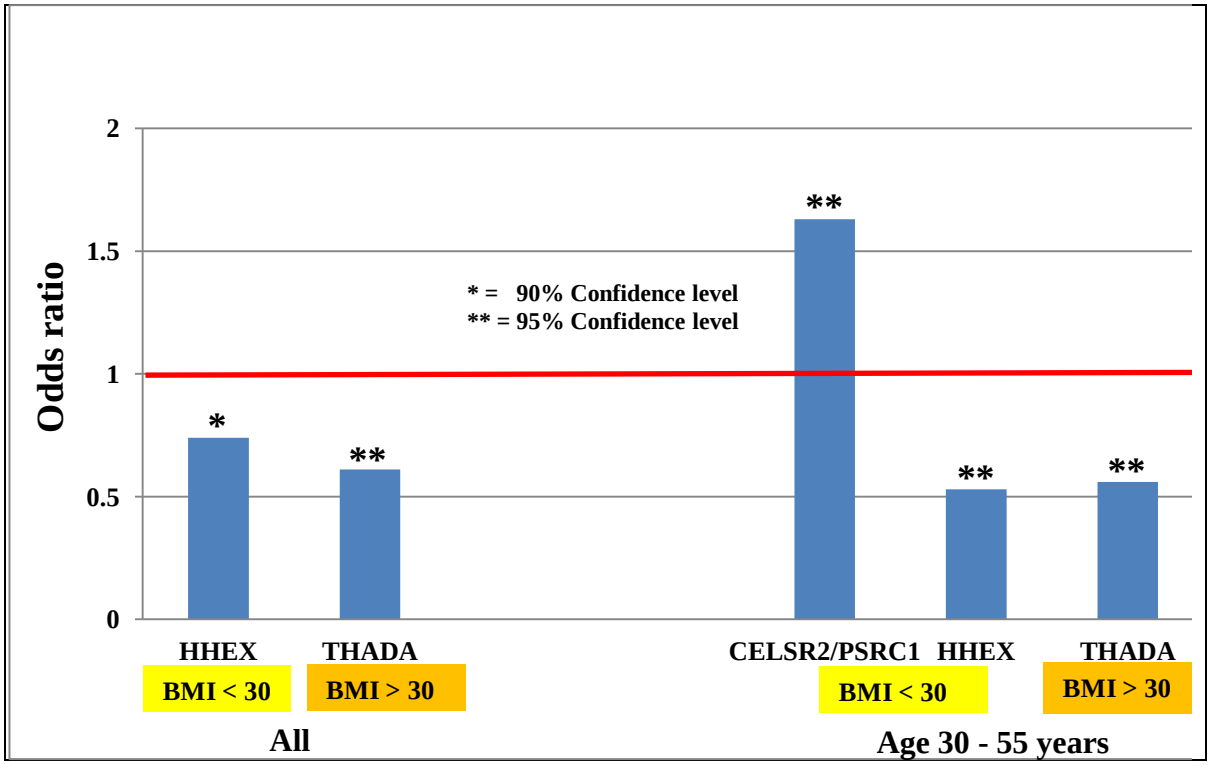

Figure 4. Influence of BMI on genetic risk of T2D diagnosis

DISCUSSION

The study of the prevalence of risk-conferring SNPs for complex chronic diseases has garnered significant interest by the medical community and Air Force Medical Service. To date this is the largest Department of Defense intramural study to examine the prevalence of diabetes-relevant SNPs among the Tricare beneficiary population.

The majority of SNPs that appeared to confer risk towards T2D diagnosis are related to either beta cell function, insulin levels during the oral glucose tolerance test, or insulin sensitivity. Beta cells (β-cells) are a type of cell produced in a hormone-producing region of the pancreas known as the islets of Langerhans. β-cells are the most populous cell in the islets and their primary function is to store and release insulin. Insulin is the hormone that brings about effects which reduce blood glucose levels. Since the primary pathophysiology of Type 2 diabetes is poor control of blood glucose control, β-cell activity is often used as an indicator of whether a newly-diagnosed diabetes patient has sufficient β-cell activity thus not requiring daily insulin injections. The preference of patients and diabetes health care providers is to delay the need for daily insulin injections through the use of oral anti-diabetes medications. However, a growing proportion of patients being treated for diabetes incur "failure to respond" to oral anti-diabetes medications suggesting. Although C-peptide (a biomarker indicative of β-cell activity) is a standard assay among newly-diagnosed diabetes patients, it is unclear if whether C-peptide is regularly included as part of the semi-annual clinical chemistry exam that measures hemoglobin A1C; an indicator of effective blood glucose control. Thus, the health care provider's knowledge of a patient's

increased or decreased *genetic* risk of β-cell dysfunction (e.g., diminished insulin levels or sensitivity) could affect short- and long-term outcomes during the treatment of diabetes as well as provide the pathophysiologic rationale to regularly monitor additional biomarkers.

Comparison of SNP Frequency between T2D Group and Military Students

The prevalence of the five SNPs that have been widely reported in the literature as being associated with insulin sensitivity or β-cell function were comparable between the non-diseased, otherwise healthy Military Student group and the group that had been diagnosed with T2D. These genes are *CDKL1, CELST2/ PSRC1, IGFBP2, JAZF1, and PPAR-γ.* This suggests a relevant interaction between the genome and the environment with respect to diabetes onset. Elements of the environment include physical activity, weight management, and diet; three factors that garner attention from every active-duty service member as they prepare for their annual physical fitness assessment. Currently, the AFMS has an interest in the onset of metabolic disorders within five years of military retirement[24]. The knowledge of the five aforementioned SNPs could be clinically useful in identifying military retirees who are at significant risk of T2D.

Increased Risk for T2D Diagnosis Conferred from SNPs

We found that a SNP in the *NOTCH2* gene conferred a risk of T2D diagnosis at all ages but not among subjects in the 30-55 year old group. Since *NOTCH2* has been associated with β-cell dysfunction, our finding suggests that the risk of T2D diagnosis conferred from a SNP in the *NOTCH2* may be a function of advancing age. Gender-specific disparities regarding risk of chronic disease and/or poor outcomes have been well-studied. Although several modifiable risk factors have been identified, the biological basis for gender disparity remains elusive. In our study we found gender-specific trends in genetic risk that were related to dyslipidemia. Specifically, a SNP in *PCSK9* (a gene that plays a critical role in low-density lipoprotein clearance) conferred a 2-fold risk of T2D diagnosis among males of all ages and over a 3-fold risk among males 30 – 55 years of age. This is significant since a SNP in the *PCSK9* gene has been reported to be associated with Familial Hypercholesterolemia or FH; a common diabetes co-morbidity and a powerful predictor of premature coronary artery disease (CAD). These findings suggest that with respect to CAD, male Tricare beneficiaries who are being treated for diabetes and are in the working adult age group may require more rigorous monitoring of their lipid profiles, response to statins, and physiological signs of CAD.

When examining increased risk among Blacks of all ages, a 2-fold risk of T2D diagnosis was conferred by a SNP in either *SLC30A8* (OR = 2.05) or *JAZF1* (OR = 2.2). When we further examined this risk among Blacks 30 – 55 years of age, the risk of T2D diagnosis conferred by a SNP in *SLC30A8* increased substantially. *SLC30A8* and *JAZF1* are each hypothesized to play critical roles in insulin secretion from the b-cells in the pancreas to the bloodstream, production of proteins that are important in the glucose metabolic pathway, and possibly β-cell dysfunction. Studies that have examined disparities in the pathophysiology of diabetes between Blacks and Whites have consistently attributed the cause to the barriers of adequate and timely health care. However, our study findings have the potential to translate into optimizing long-term diabetes management (that includes awareness of risk for accelerated loss of b-cell function and/or adequate glucose metabolism) among Blacks who have been recently diagnosed with T2D. Lastly, a SNP in the gene known as *CELSR2/PSRC1* was found to confer an increased risk of

T2D diagnosis among those who were not obese and were within the 30 – 55 year age group. *CELSR2/PSRC1* has become increasingly important in research regarding diabetes management since it is associated with increasing levels of low-density lipoproteins in the serum; a powerful risk factor for coronary artery disease and a clinical indicator of a common co-morbidity among patients being treated for T2D. Furthermore, in a follow-up analysis we found that this SNP was most prevalent in the Military Student population. The implication of the risk conferred by a SNP in *CELSR2/PSRC1* suggests a significant role of lipid metabolism and possibly diet in the risk of T2D.

Inverse Risk for T2D Diagnosis Conferred from SNPs

Our analysis also revealed several SNPs that were less likely to be present in the T2D group. The SNPs that were found to confer such inverse risk among subgroups in our study were *HHEX* (a gene that is associated with insulin sensitivity), *THADA* (a gene shown to affect the 2-hour insulin level during an oral glucose tolerance test), and *PPAR-γ* (a gene that influences the patient's response to a popular class of anti-diabetes drugs known as glitazones). Our study findings concur with others that have failed to find an association between *THADA* and T2D diagnosis despite the fact that this gene plays a critical role in 2-hour insulin response during the oral glucose tolerance test. Most interesting is that several studies have found difficulty concurring on the risk conferred by a SNP in *PPAR-γ* in spite of its well-characterized role in improving insulin sensitivity. However, the continuous discovery of variants in mutations of the PPAR-γ gene may suggest that several PPAR-γ SNPs may work in concert to confer risk of T2D.

Clinical Utility of Study Findings within the Context of AFMS Genomic-Based Medicine

Our study findings exemplify how the translation of biomedical research to clinical care has the power to optimize long-term diabetes management especially among those who have who have been recently diagnosed with T2D. For example, it remains unknown whether patients who do not possess a SNP in the *HHEX* gene are more likely to incur a greater degree of insulin resistance. Another example is the inverse risk conferred by a SNP in *THADA;* a SNP is shown to affect the 2-hour insulin level during an oral glucose tolerance test. Although the "shuttling" of insulin from the b-cells to the blood stream appears to be influenced by *THADA*, it is unclear if whether impaired insulin shuttling is an indicator of β-cell dysfunction or disruption in cell signalling specific to insulin movement from the pancreas to the bloodstream. An example of such would be the patient who performs well during an oral glucose tolerance test (indicating sufficient β-cell activity) but still incurs bouts of insulin resistance or hyperinsulinemia.

Study Limitations

As previously mentioned, this was the first study to assess the genetic risk towards Type 2 Diabetes diagnosis among Tricare beneficiaries. A limitation of this study was that we did not collect information regarding length of disease or age of diagnosis. Future enrollees will be asked this information since there is much debate about whether early-adult onset T2D (e.g., < 40 years of age) is more aggressive than late onset (e.g., > 60 years of age). We feel that such information in combination with the patient's genotype would be clinically useful in screening young adult patients who may also be "at risk" for metabolic syndrome and premature cardiovascular disease. Other information to be collected from future study enrollees include

abdominal obesity, triglyceride/lipid profiles, and family history (specifically maternal history of metabolic disease).

Future Directions

This study protocol was approved to enroll up to subjects and designed/funded for analysis of 2,000 subjects (1,000 diabetics, 500 controls and 500 students). Although we initially incurred enrollment limitations, we feel that a future directions may include using a two-stage case-control association test (ideally genotyping at least 100 controls) to improve our chances of detecting a statistically significant association between the selected 17 SNPs and the diagnosis of T2D. Further statistical analysis will include testing for Hardy-Weinberg Equilibrium for possible exclusion from analysis. Further study of the associations between SNPs will be investigated using multiple R packages (hapassoc, haplo.stats, haplo.ccs) designed for haplotype estimation to also include the creation of an LDHeatmap, a graphical display of linkage disequilibria between SNPs. Additionally, the inclusion of disease indicators in our risk model as well as the interaction of two or more SNPs are encouraged and will be examined during the upcoming months.

CONCLUSION

This study is an example of evidence-based medicine that serves as the foundation of for the Air Force's Patient Centered Precision Care program; a personalized medicine approach to disease management. We identified five SNPs that warrant further study as potential biomarkers for incorporation into a risk characterization profile for personalized medicine program. Additional follow-on studies will be proposed using genomic markers to characterize risk level as a predictive tool to identify individuals who may benefit from lifestyle modification and intervention, using tools such as the Diabetes Prevention Program.

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